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Bioavailable and biodegradable dissolved organic nitrogen in activated sludge and trickling filter wastewater treatment plants

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ABSTRACT

A study was carried out to understand the fate of biodegradable dissolved organic nitrogen (BDON) and bioavailable dissolved organic nitrogen (ABDON) along the treatment trains of a wastewater treatment facility (WWTF) equipped with an activated sludge (AS) system and a WWTF equipped with a two-stage trickling filter (TF) process. A mixed culture bacterial inoculum was used for BDON determination, while a pure cultured algal inoculum (*Selenastrum capricornutum*) and a combination of the bacterial and alga inocula were used for ABDON determination. Results show that BDON and ABDON varied significantly within the treatment facility and between the two facilities. From after primary clarification to final effluent, the TF facility removed 65% of BDON and 63% of ABDON while the AS facility removed 68% of BDON and 56% of ABDON. For the TF facility, BDON and ABDON were 62% and 71% of the effluent dissolved organic nitrogen (DON), while they were 26% and 47% of the effluent DON for the AS WWTF. BDON and ABDON results, which are based on incubation of samples under different inocula (bacteria only, algae only, and bacteria + algae), further showed that some portions of DON are utilizable by bacteria only or algae only while there is a portion of DON utilizable by either bacteria or algae. DON utilization was the highest when both bacteria and algae were used as a co-inoculum in the samples. This study is the first to investigate the fate of BDON and ABDON along the treatment trains of two different WWTFs.

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1. Introduction

Current regulations for total nitrogen (TN) in wastewater treatment facility (WWTF) effluents in many parts of the United States are approaching 5 mg N/L or less to control eutrophication and hypoxia conditions in estuaries and bays. With recent advances in nutrient removal technologies,

WWTFs are able to achieve high inorganic nitrogen removal, leading to dissolved organic nitrogen (DON) being a major nitrogen form (>50%) of the effluent total dissolved nitrogen (TDN). Parkin and McCarty (1981) reported that about 70% of the total influent DON can be removed in suspended growth systems of WWTFs. Simsek et al. (2012) reported that 62% of the influent DON was removed by a trickling filter (TF)

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treatment process. The effluent DON was 50% and 51% biodegradable (ammonifiable) for activated sludge (AS) and TF WWTFs, respectively (Murthy et al., 2006; Simsek et al., 2012).

Traditionally effluent DON was believed to be fully non-biodegradable and hence would not be available as a nutrient source in receiving waters. However, recent studies have shown that effluent DON comprises of various forms of organic nitrogen that can be bioavailable to natural algae and plankton (Pehlivanoglu and Sedlak, 2004; Sattayatewa et al., 2009; Filippino et al., 2011). DON in treated effluent plays an important role in nitrogen cycling. Some forms of DON such as free amino acids can be readily bioavailable for a direct algal uptake while some forms are bioavailable after bacterial degradation (Pehlivanoglu and Sedlak, 2004; Bronk et al., 2007). DON can become more bioavailable to algae through hydrolysis and/or mineralization (to NH_4^+ or NO_3^-) by bacteria. Biodegradable DON (BDON) is a portion of DON that can be mineralized by an acclimated mixed bacterial culture (Khan et al., 2009) while bioavailable DON (ABDON) is a fraction of DON that is directly or indirectly available as a nitrogen source for aquatic plant species (Pehlivanoglu and Sedlak, 2004; Pehlivanoglu-Mantas and Sedlak, 2006; Sattayatewa et al., 2009).

Studies on BDON and ABDON in wastewater effluent and aquatic environment have been conducted (Pehlivanoglu and Sedlak, 2004; Murthy et al., 2006; Khan et al., 2009; Sattayatewa et al., 2009; Bronk et al., 2010; Filippino et al., 2011; Simsek et al., 2012). However, there has been no study available on BDON and ABDON in a wastewater treatment train. Sattayatewa et al. (2009) determined BDON and ABDON in the effluent from a 4-stage Bardenpho process. They used mixed liquor suspended solids (MLSS) and a pure culture alga *Selenastrum capricornutum* as inocula for BDON and ABDON measurements, respectively. Also, a combined bacterial and algal inoculum was used for ABDON determination. They reported that about 28–57% of the effluent DON was bioavailable or biodegradable regardless of the type of the test species used. DON reduction rate during the ABDON incubation with the combined inoculum was 0.13 day^{-1} compared to 0.04 day^{-1} during the BDON incubation. Sattayatewa et al. (2009) further reported that there was a symbiotic relationship between algae and bacteria and this could shorten the incubation time for the ABDON procedure. Other studies also obtained the same result on the relationship between algae and bacteria in ABDON procedures (Pehlivanoglu and Sedlak, 2004; Urgun-Demirtas et al., 2008).

Bioavailability of DON (ABDON/DON) of the effluents from AS WWTFs to algae can be as high as 40% while that to algae and bacteria together is up to 60% (Urgun-Demirtas et al., 2008). Previous studies on effluent DON bioavailability, however, used the samples from WWTFs that are equipped with TDN removal technologies (Pehlivanoglu and Sedlak, 2004; Bronk et al., 2010; Filippino et al., 2011). The ABDON in the effluents from TF WWTFs has not been studied. In addition, previous studies on ABDON (Pehlivanoglu and Sedlak, 2004; Bronk et al., 2010; Filippino et al., 2011) did not focus on its fate through various stages of WWTFs.

Simsek et al. (2012) examined the fate of BDON through a full-scale two-stage trickling filter WWTF. BDON was removed mainly by the trickling filters (both stages). Average BDON

removal efficiency by the entire treatment facility and final effluent BDON concentration were 72% and 1.80 mg N/L. DON biodegradability (BDON/DON) for raw wastewater samples and samples from all treatment units varied from 51% to 69%. Other than the work by Simsek et al. (2012), there has been no study available on a BDON profile along a WWTF particularly one with activated sludge process. The fate of ABDON through a WWTF has never been investigated. Knowledge on the fate of BDON and ABDON along treatment train helps to understand the roles of WWTP treatment units in the removal of these different types of nitrogen. The objective of this study was to determine the fate of BDON and ABDON along the treatment trains of a WWTF equipped with an AS system and a WWTF equipped with a TF system. It should be noted that the fate of BDON through a TF WWTF was investigated again to compare the results with ABDON values based on the same samples. Additionally, DON and BDON profiles along the AS and the TF WWTFs were simulated using a wastewater modeling software, BioWin[®]. Plant operational data and measured dissolved nitrogen species (ammonia, nitrite, nitrate, DON, and BDON) for both the facilities were used during the model setup, calibration and verification purposes.

2. Materials and methods

2.1. Sample sources

Samples were obtained from two different treatment plants, which are the City of Fargo WWTF (Fargo, ND, USA), and the City of Moorhead WWTF (Moorhead, MN, USA). The Fargo WWTF has a peak pumping capacity of $110,000 \text{ m}^3 \text{ day}^{-1}$ and an average flow of $57,000 \text{ m}^3 \text{ day}^{-1}$. The Moorhead WWTF has a peak pumping capacity of $38,000 \text{ m}^3 \text{ day}^{-1}$ and an average flow of $15,000 \text{ m}^3 \text{ day}^{-1}$. Both plants have to comply with the discharge limits for biochemical oxygen demand (BOD) and ammonia (based on the receiving river flow rate) but are not subject to any TN or total phosphorus limits. Both plants are not regulated for fecal coliform in the winter months (November to March) and therefore do not chlorinate and dechlorinate during that period.

2.2. Facility description

2.2.1. Fargo wastewater treatment facility

The City of Fargo WWTF mainly treats the wastewater for BOD and ammonia through a two-stage trickling filter process (Fig. 1). The treated effluent is either discharged to the Red River or pumped to stabilization ponds when flood conditions exist. The treated wastewater is stored in these ponds until it can be discharged to the Red River. Settled solids from intermediate and final clarifiers are brought back to the head of the plant. Settled solids from the primary clarifiers are further treated by 3 anaerobic digesters followed by dewatering in sand drying beds or belt filter presses. The stabilized biosolids are hauled to a city landfill for disposal.

2.2.2. Moorhead wastewater treatment facility

The City of Moorhead WWTF treats the wastewater for BOD and ammonia through high purity oxygen activated sludge

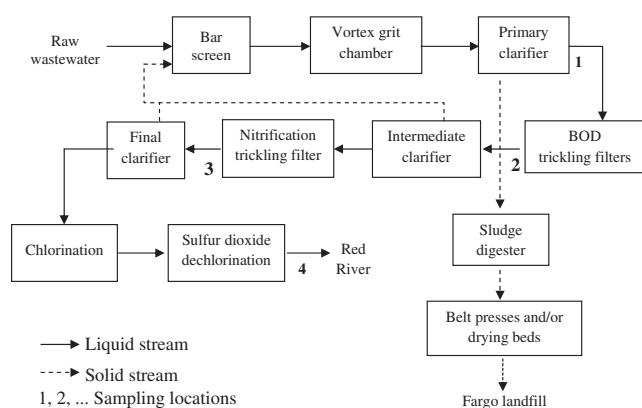


Fig. 1 – A simplified schematic diagram of the City of Fargo WWTF.

(HPO-AS) and moving bed biofilm reactor (MBBR) (Fig. 2). The hydraulic and solids retention times of the HPO-AS are 7 h and 3 days while those of the MBBR are 6 h and 32 days, respectively. The facility discharges its final effluent to the Red River. Settled solids from primary and secondary clarifiers are dewatered through dissolved air flotation thickeners and stabilized using anaerobic digesters. The treated biosolids are stored in a biosolids storage tank and land applied during the summer months. Decanted supernatant from the thickeners is sent back to flow equalization basins at the head of the plant.

2.3. Sample collections, and sources and preparations of inocula

Grab samples were collected from four different locations along the treatment train of the Fargo WWTF (Fig. 1) and the Moorhead WWTF (Fig. 2). The samples were collected on a monthly basis for five months during the winter season from both plants. One liter sample was collected from each sampling location from both WWTFs.

Bacterial inocula for BDON determination were collected from the Fargo and Moorhead WWTFs and used to inoculate their respective samples. Raw wastewater sample was used as a bacterial inoculum for the Fargo WWTF samples. The Fargo WWTF recycles settled solids from intermediate and final

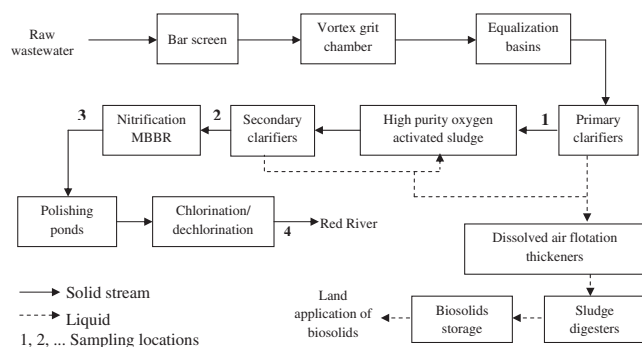


Fig. 2 – A simplified schematic diagram of the City of Moorhead WWTF.

clarifiers and hence, the influent wastewater contains a representation of mixed bacterial culture in the treatment facility. For the samples from the Moorhead WWTF, diluted MLSS (10 fold dilution of approximately 2,500 mg suspended solids/L) were used as a bacterial inoculum. Cultivation and maintenance of *S. capricornutum* (UTEX, University of Texas Culture Collection of Algae, Austin, TX, USA) were performed according to the instruction provided by the culture manufacturer (UTEX, 2011).

2.4. DON, BDON and ABDON determination procedures

Samples were analyzed for DON, BDON and ABDON. Each sample was filtered through a 0.2 µm pore-size hydrophilic polyethersulfone membrane filter (Pall Co., Port Washington, NY, USA) immediately after collection. Samples with high concentrations of total solids (mainly primary clarifier effluent) were initially filtered through a 1.2 µm pore-size Whatman glass microfiber filter (Whatman Inc., Kent, UK) before filtering through the 0.2 µm pore-size membrane filter. The filtered samples were autoclaved for 15 min to remove any remaining bacteria. About 50 mL of the autoclaved sample were used for dissolved ammonia N ($\text{DNH}_3\text{-N}$), dissolved nitrite N ($\text{DNO}_2\text{-N}$), dissolved nitrate N ($\text{DNO}_3\text{-N}$), and TDN analyses and the results were used for calculating DON before incubation (DON_i) according to Equation (1).

The BDON and ABDON procedures rely on the change of DON in the sample before (DON_i) and after (DON_f) a 28-day incubation period. DON after the incubation was determined in the same manner as DON before the incubation (Equation (1)). A seed control (sample b) was prepared for each bioassay by adding the inoculum to distilled deionized water and treating it the same way as the sample (DON_{bi} and DON_{bf}). BDON and ABDON were calculated according to Equation (2).

$$\text{DON} = \text{TDN} - \text{DNH}_3 - \text{N} - \text{DNO}_2 - \text{N} - \text{DNO}_3 - \text{N} \quad (1)$$

$$\text{BDON or ABDON} = [(\text{DON}_i - \text{DON}_f) - (\text{DON}_{bi} - \text{DON}_{bf})] \quad (2)$$

The entire BDON procedure is described in Simsek et al. (2012). There are slight differences between the BDON and ABDON procedures. The BDON procedure requires incubation in the dark to control algal growth, while ABDON determination requires algal growth and hence, the incubation is conducted under artificial light (two cool-white fluorescent light bulbs, 23 W and 380 mA each) with 12 h light and 12 h dark cycles. The light intensity during the 12 h light cycle was 770 lux (HOBO U12-012 temp/RH/light external data logger, Onset Computer Corporation, Bourne, MA, USA). BDON was performed in 250 mL amber bottles while ABDON was performed in 250 mL clear glass bottles. In BDON determination, 2 mL mixed culture bacterial inoculum are used (Khan et al., 2009), while in ABDON determination the samples were seeded with 5 mL pure culture algae (*S. capricornutum*) or 2 mL mixed culture bacteria + 5 mL *S. capricornutum* (Pehlivanoglu and Sedlak, 2004; Urgun-Demirtas et al., 2008; Sattayatewa et al., 2009). For all inoculation conditions for BDON and ABDON, the sample volume was 200 mL and the incubation period was 28 days. Both BDON and ABDON procedures were performed at 20 °C. All the ABDON samples were agitated using an orbital shaker at 80 rpm during the incubation.

2.5. Analytical methods

The salicylate, diazotization, and second derivative ultra-violet spectrophotometric (SDUS) methods were used to determine ammonia, nitrite, and nitrate according to Hach Methods #10023 (0.02 and 2.50 mg/L as NH_4^+-N) and #10031 (0.04 and 50 mg/L as NH_4^+-N), Hach Method #10019, and APHA (2005), respectively. The diazotization method is suitable for a low range of nitrite concentration (0.003 and 0.5 mg/L as NO_2^--N). Samples with high nitrite concentrations (2 and 250 mg/L as NO_2^--N) were analyzed by the ferrous sulfate method (Hach Method #8153). For TDN determination, all N species in the sample were converted to nitrate via modified persulfate digestion (Sattayatewa and Pagilla, 2008) and then the SDUS method was used to determine nitrate. All the parameters were determined in duplicate or triplicate and average values were reported. All the glassware was washed with soap, rinsed with tap water, kept in a 5% v/v hydrochloric acid bath overnight and rinsed with de-ionized water and then autoclaved before use.

2.6. Statistical analyses and modeling strategy

Two-way analysis of variance (ANOVA) was performed using the “MIXED” procedure in SAS (SAS Version 6.1; SAS Institute, Cary, NC) to determine the statistical differences in 1) initial DON (before incubation) and DON after incubation under different seed types, and 2) BDON, ABDON (algae seeded samples), and ABDON (algae + bacteria seeded samples). A split plot design was used in which the main-plot factor was sampling location and the subplot factor was DON, BDON or ABDON. A “LSMEANS” option in the “MIXED” procedure was used to make a pairwise comparison. The samples were collected five times on different dates, which were the replications in ANOVA. The compared values are considered statistically different when $p < 0.05$.

BioWin® version 3.1 (Envirosim Associates, Ltd.) was used to simulate DON and BDON profiles along the Fargo and Moorhead WWTFs. Influent fractionation was performed using historical plant data. A calibrated model from a previous study by Simsek et al. (2012) was used to simulate various nitrogen species through the Fargo wastewater treatment processes. A separate calibration was performed for the data collected from the Moorhead WWTF.

Historical plant sampling data were used for influent wastewater characterization and fractionation calculations. Models for each treatment plant were configured using physical characteristics of treatment units, influent fractionation information, and influent wastewater characteristics. The default BioWin kinetic and stoichiometric parameters were utilized during the initial calibration steps. The calibration was a trial and error method to match the model simulated BOD, chemical oxygen demand (COD), DNH_3-N , DNO_2-N , DNO_3-N , total Kjeldahl nitrogen (TKN), TDN, DON and BDON with the measured data. Detailed calibration procedures are described in Simsek et al. (2012). All calibrations and simulations were performed based on steady state conditions.

3. Results and discussion

The profiles of TDN, DON, BDON, ABDON, and selected ratios of these parameters along the treatment trains of the Fargo and Moorhead WWTFs are presented in Figs. 3–6. The data and error bars are based on averages and standard deviations for 5 samples collected from 5 different months.

3.1. Fargo WWTF

3.1.1. Inorganic nitrogen species and TDN

After the incubation, almost all of the ammonia was nitrified (remaining ammonia < 0.30 mg N/L) in all the samples seeded with bacteria and bacteria + algae while only the samples from nitrification trickling filters and secondary clarifiers were almost completely nitrified when seeded with algae alone (data not shown). The remaining ammonia concentrations after the incubation with algae seed alone averaged 10.32 and 4.24 mg N/L for the samples from the primary clarifiers and BOD trickling filters, respectively. This remaining ammonia in the sample is an indication that algae itself could not utilize the ammonia completely during the incubation.

Average nitrite concentration in all the samples before incubation was very low (< 0.40 mg N/L, data not shown). After the incubation, nitrite concentrations for all the samples

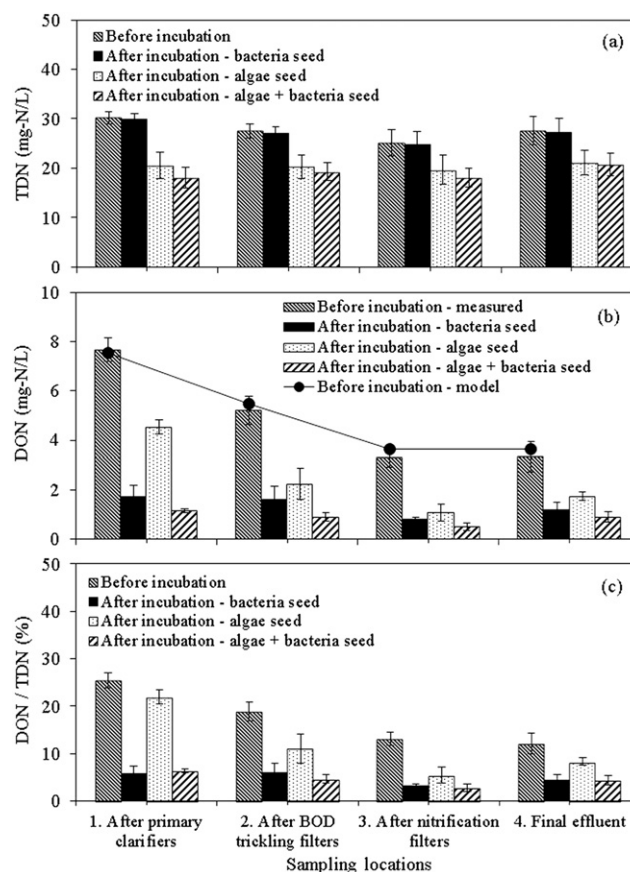


Fig. 3 – (a) TDN before and after incubation, (b) DON before and after incubation, (c) DON as a percentage of TDN before and after incubation for samples through the treatment train of the City of Fargo WWTF.

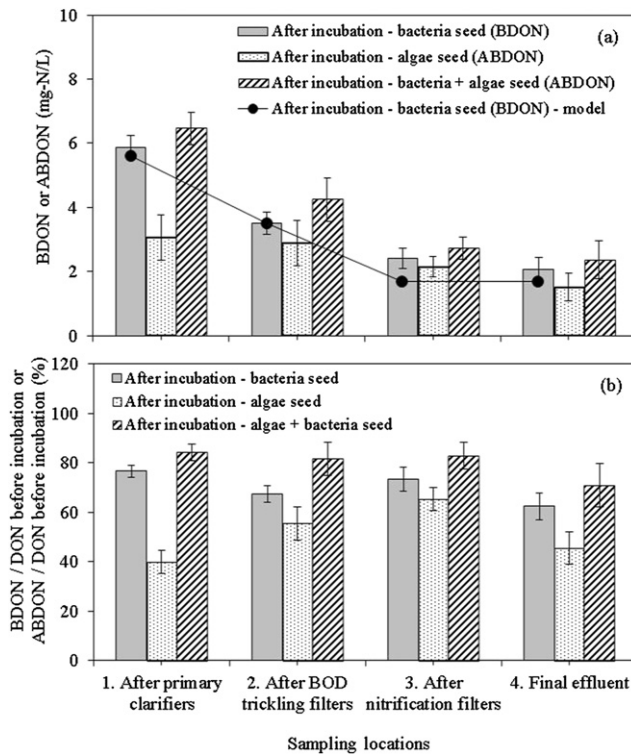


Fig. 4 – (a) Effluent BDON and effluent ABDON, (b) Effluent BDON as a percentage of effluent DON, effluent ABDON as a percentage of effluent DON for samples through the treatment train of the City of Fargo WWTF.

(seeded with bacteria, algae, or bacteria + algae) from nitrification trickling filters and secondary clarifiers were also low; almost all of the ammonia was nitrified all the way to nitrate. For the samples from the BOD trickling filters, nitrate was a major form of nitrogen after the incubation. There were low concentrations of nitrite (1.41–5.82 mg N/L) in the BOD trickling filters samples after the incubation regardless of the inoculum type. Nitrite was high after the incubation for the primary clarifier samples seeded with bacteria only and algae + bacteria (23.97 and 14.95 mg N/L). A reason for this high nitrite was because DO was not adequate during the incubation to nitrify all the way to nitrate. It should be noted that this partial nitrification had no effect on DON and BDON results.

TDN concentration in the bacteria seeded samples was quite balanced before and after incubation for all the sampling locations (Fig. 3a). Substantial discrepancies in TDN between before and after the incubation for the samples seeded with algae only and algae + bacteria were likely due to the uptake of nitrogen by algae which was much more than the uptake by bacteria. TDN after incubation was slightly lower in the algae + bacteria seeded samples compared to the algae seeded samples in all locations. However, judging from the standard deviations (error bars) associated with the data, it is very likely that they are not statistically different.

3.1.2. Dissolved organic nitrogen

DON profiles for the samples collected from the Fargo WWTF are presented in Fig. 3b for both before and after incubation.

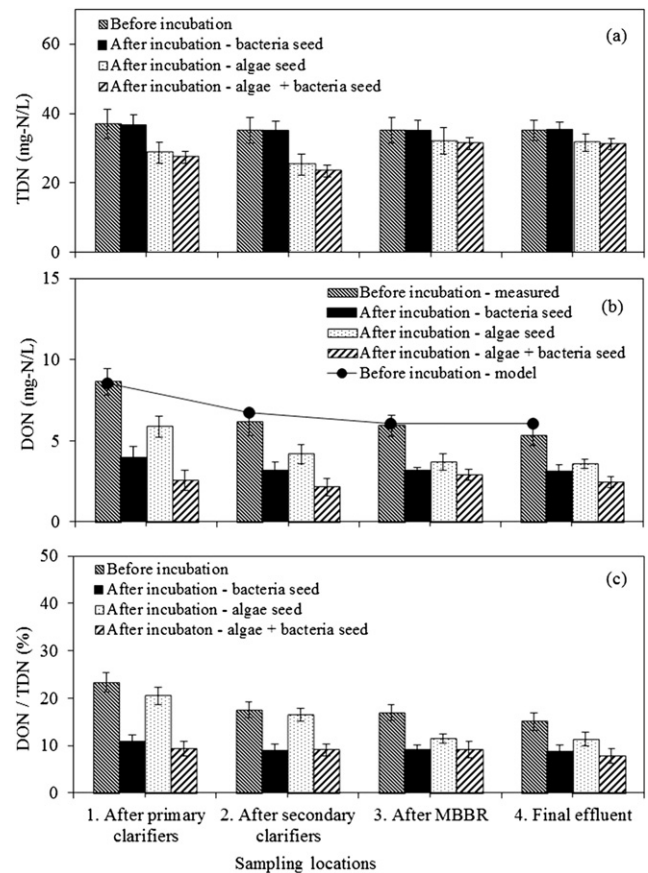


Fig. 5 – (a) TDN before and after incubation, (b) DON before and after incubation, (c) DON as a percentage of TDN before and after incubation for samples through the treatment train of the City of Moorhead WWTF.

Before the incubation, DON averaged at 7.67 mg-N/L in the samples from primary clarifiers and 3.33 mg N/L in the final effluent corresponding to 57% removal of DON from primary effluent by the WWTF. Two-way ANOVA results showed that initial DON (before the incubation) was statistically different from DON after incubation for all sampling locations. For the algae seeded samples, the remaining DON concentrations in the samples after incubation were higher than those in the bacteria only and bacteria + algae seeded samples. This result showed that bacteria can ammonify and uptake (in combination) more DON than algae alone.

The highest DON reduction during the incubation, indicating the highest DON bioavailability or the lowest DON recalcitrance, was observed in the samples inoculated with algae + bacteria for all samples (Fig. 3b). Previous research showed a similar increase in effluent DON bioavailability when algae and bacteria were presented together compared to algae alone or bacteria alone (Pehlivanoglu and Sedlak, 2004; Urgun-Demirtas et al., 2008). A symbiotic relationship between algae and bacteria increased DON utilization and therefore reduced the recalcitrant DON concentration in the samples. This suggests that for water environment receiving treated wastewater, more growth of algae (potential eutrophication) might be observed if more bacteria are present.

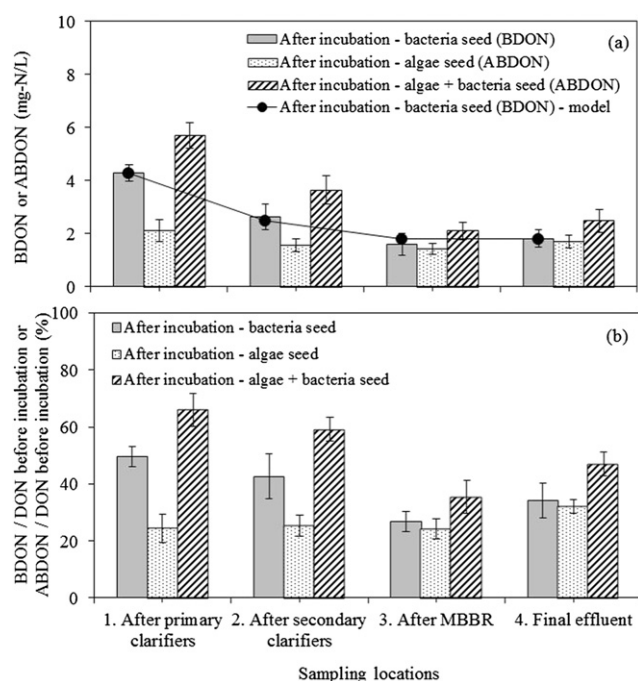


Fig. 6 – (a) Effluent BDON and effluent ABDON, (b) Effluent BDON as a percentage of effluent DON, effluent ABDON as a percentage of effluent DON for samples through the treatment train of the City of Moorhead WWTF.

The DON/TDN fraction data (Fig. 3c) are very similar to the DON data (Fig. 3b). For the samples seeded with algae only, less DON/TDN reduction was observed during the incubation confirming limited ability of the algae to ammonify DON regardless of DON level and characteristics. Similarly, Urgun-Demirtas et al. (2008) found that only 21% of initial DON in denitrified effluent was bioavailable to algae (*S. capricornutum*) during a 14-day incubation period. Pehlivanoglu and Sedlak (2004) conducted a similar study using denitrified effluent with algae (*S. capricornutum*) and/or bacteria inocula and concluded that wastewater-derived DON was not bioavailable to algae (*S. capricornutum*) in the absence of bacteria. In this study, about 40–84% of the DON from all four locations in the Fargo WWTF was biodegradable or bioavailable to the test species. DON reduction in algae + bacteria seeded samples was the highest for all four locations.

3.1.3. Biodegradable and bioavailable dissolved organic nitrogen

BDON and ABDON concentrations decreased along the treatment train of the Fargo WWTF (Fig. 4a). BDON and ABDON removal occurred mainly in the trickling filters except for ABDON (algae seed) which was substantially removed by the secondary clarifiers as well. From after primary clarifier to final effluent, BDON, ABDON (algae seed), and ABDON (algae + bacteria seed) removal efficiencies were 65%, 51%, and 63%, respectively. As expected, the order of BDON and ABDON exertions in all samples was as follows: ABDON in algae + bacteria seeded samples > BDON (bacteria seeded samples) > ABDON in algae seeded samples. Bacteria and algae together uptake and ammonify DON more than only

algae or only bacteria seeds. Bacteria break down DON to lower molecular weight compounds and subsequently, algae can utilize some of those compounds (Carlsson et al., 1999; Pehlivanoglu and Sedlak, 2004). The statistical analysis showed that BDON, ABDON (algae only seeded), and ABDON (bacteria + algae seeded) were not different for the last three sampling locations even though Fig. 4a shows the algae only seeded ABDON was the lowest and the bacteria and algae seeded ABDON was the highest in these locations. Statistically, BDON and ABDON (bacteria + algae seeded) were not different in the after primary clarifier samples but the algae only seeded ABDON was different from BDON and ABDON (bacteria + algae seeded).

Identifiable effluent DON usually accounts for less than 10% of DON and a major portion of DON most probably consists of polymerized biological compounds (Pehlivanoglu-Mantas and Sedlak, 2006). Previous studies showed that free amino acids, urea, and nucleic acids in DON are identifiable portions of DON and are taken up readily by bacteria and/or algae (Pehlivanoglu and Sedlak, 2004; Pehlivanoglu-Mantas and Sedlak, 2006; Urgun-Demirtas et al., 2008). The algae and bacteria are in competition for nitrate when nitrate is the only nitrogen source in the system. Bacteria use nitrate to support their growth and therefore bioavailable nitrogen source for algae decreases. These previous studies and this work show that in the presence of both nitrate and DON in the system, bacteria increase the bioavailability of nitrogen to algae since bacteria degrade DON. This reiterates the importance of bacteria on algal growth in receiving water.

Based on the BDON and ABDON results from different inoculum conditions, it is possible to identify whether algae and bacteria were utilizing (uptaking and ammonifying in combination) the same or different fractions of DON using Equation (3). Overlapping DON is DON that can be uptaken and ammonified by either bacteria or algae.

$$\begin{aligned} \text{Overlapping DON} = & [\text{ABDON}(\text{algae seed only}) \\ & + \text{BDON}(\text{bacteria seed only})] \\ & - \text{ABDON}(\text{algae + bacteria seed}) \end{aligned} \quad (3)$$

If algae and bacteria were utilizing totally different fractions of DON, overlapping DON should be zero (no overlap between DON utilized by algae and DON utilized by bacteria). Equation (3) is valid because the samples were filtered and autoclaved before the re-inoculation and incubation. The incubation with bacteria seed only was in the dark (to prevent algal growth) while the DON reduction for algal seed only was always lower than bacterial seed only suggesting that there is no bacterial contamination. Overlapping DON can also be used to indicate relative potential for symbiotic relationship between algae and bacteria. More overlapping DON suggests less potential for the symbiosis.

The overlapping DON was calculated for all the samples and the results are presented in Table 1. There was overlapping DON in all the samples indicating that there is a common portion of DON that can be utilized by either algae or bacteria. The overlapping DON was lower than BDON and ABDON (algae seed only) suggesting that there were portions of DON that can be used strictly by bacteria and strictly by algae which can be calculated by subtracting overlapping DON

Table 1 – Overlapping DON and DON utilizable exclusively by algae and bacteria for samples from Fargo WWTF.

Sample location	Overlapping DON (mg/L)	DON in mg/L utilizable exclusively by	
		Bacteria	Algae
After primary clarifier	2.49	3.39	0.58
After BOD trickling filters	2.15	1.36	0.74
After nitrification trickling filters	1.84	0.58	0.31
Final effluent	1.23	0.85	0.29

from BDON and ABDON (algae seed only), respectively. These portions of DON which exist for both bacteria and algae are also shown in Table 1. DON utilizable exclusively by bacteria was higher than by algae for all the samples indicating more versatility for bacteria in going after different DON species for ammonification and uptake in combination. These results suggest the benefit of having both types of seed for sample inoculation as it would predict the maximum DON that could support algae growth directly and indirectly (through ammonification by bacteria).

BDON/(DON before incubation) and ABDON/(DON before incubation), also known as DON biodegradability and bioavailability, of the samples are presented in Fig. 4b. In general, biodegradability and bioavailability (algae and bacteria seeded samples) tended to decrease slightly through the treatment train. The DON biodegradability trend is similar to that observed by Simsek et al. (2012). There is no conclusive trend on DON bioavailability based on algae only seed (Fig. 4b). Although the treatment units reduced BDON and ABDON, they also removed non-BDON and non-ABDON resulting in limited changes in DON biodegradability and bioavailability among the samples. In addition, it should be noted that the fractions of BDON and ABDON of the final effluent DON (DON biodegradability and bioavailability) are still quite high (45–70%) implicating that major portions of discharged DON are biodegradable and bioavailable which are not good for receiving waters.

Based on the BDON and ABDON results, to minimize BDON and ABDON discharged to receiving waters, algae should be used along with bacteria in wastewater treatment particularly in polishing treatment units. As indicated in Table 1, the concentrations of DON utilizable exclusively by algae in the samples from nitrification trickling filters and final effluent were 11% and 12% of ABDON exerted by bacteria + algae seed (all three columns in Table 1 combined). With no algae based treatment, these DON concentrations will contribute to N load as well as support algal bloom in receiving waters. These results also suggest that traditional bacteria based treatment will not be able to completely remove the portion of DON that can support eutrophication.

3.2. Moorhead WWTF

3.2.1. Inorganic nitrogen species and TDN

Average ammonia concentration (data not shown) after primary clarifier of the Moorhead WWTF was 27.99 mg N/L.

A small decrease in ammonia concentrations (5%) was typically observed in the samples collected after secondary clarifiers. The HPO-AS process in the WWTF does not remove ammonia due to the toxicity of high oxygen concentration to the nitrifying microorganisms (Uemoto et al., 2000) as well as the low SRT. When there was no spike in ammonia load due to the recycling of the thickener supernatant, the MBBR normally nitrified > 90% of ammonia in the secondary effluent and the average ammonia concentration in the final effluent was <2.30 mg N/L. Similar to the Fargo WWTF results, ammonia in all the samples was totally nitrified during the incubation except for the samples seeded with algae only.

Nitrite concentrations in all samples were low (<0.10 mg/L, data not shown). For the samples from primary and secondary clarifiers which have high ammonia concentrations, partial nitrification to nitrite was observed during the incubation for some inoculum conditions. Same as described above for the trickling filter plant, inadequate oxygen recharge during the incubation was the reason for this partial nitrification. Nitrite concentrations after the incubation in the MBBR and final effluent samples were low because of full nitrification in the MBBR and during the incubation. Corresponding to nitrite concentrations, nitrate concentrations were low for primary and secondary clarifier samples and high for MBBR and final effluent samples (data not shown).

TDN profiles for the samples collected along the treatment train are shown in Fig. 5a. They show the same trend as the data for the Fargo WWTF. TDN concentrations in the only bacteria seeded samples were quite balanced before and after the incubation for all the sampling locations. Very minimal TDN was removed by the HPO-AS while there was no removal by the MBBR process. For the samples that had algae in the seed, TDN concentrations were always lower compared to bacteria seeded samples confirming that algae used more nitrogen for their growth.

3.2.2. Dissolved organic nitrogen

DON profiles for the samples collected from the Moorhead WWTF for both before and after the incubation are presented in Fig. 5b. The Moorhead WWTF samples had a higher range of DON concentrations compared to the Fargo WWTF samples (5.30–8.64 mg/L versus 3.33–7.67 mg/L). The Moorhead WWTF removed 39% of DON with the highest DON removal observed in HPO-AS at 29%. The MBBR process removed very minimal DON at 4%. The Moorhead WWTF was less efficient in removing DON than the Fargo WWTF. Two-way ANOVA results showed that DON before incubation was statistically different from DON after incubation for all sampling locations.

After the incubation, the remaining DON in the algae seeded samples was always higher than the bacteria and algae + bacteria seeded samples for all locations. The lowest remaining DON was observed in algae + bacteria seeded samples for all locations confirming that algae and bacteria together can utilize more DON than bacteria only or algae only. About 25–66% of the DON from all four sampling locations were biodegradable or bioavailable regardless of the type of the inocula.

DON fraction in TDN gradually decreased along the treatment train (Fig. 5c). About 15% of TDN in the final effluent was DON which was slightly higher than the value for the Fargo

WWTF (12%). DON to TDN ratio after the incubation for bacteria seeded samples and bacteria + algae seeded samples did not vary that much across the sample locations ranging from 8% to 11%. For the algae seeded samples, it dropped from 22% to 8% through the plant. These trends are similar to those of the Fargo WWTF.

3.2.3. Biodegradable and bioavailable dissolved organic nitrogen

BDON and ABDON data for the Moorhead WWTF are presented in Fig. 6a. ABDON for algae + bacteria seeded samples were always higher than BDON and ABDON (algae seeded only), same as the trend observed for the Fargo WWTF samples. However, the statistical analysis showed that bacteria + algae seeded ABDON was higher than the algae only seeded ABDON for all sampling locations while it was different from BDON only for the first two sampling locations. BDON and ABDON were removed primarily by HPO-AS and MBBR units. BDON and ABDON (algae seed + bacteria seed) were removed more than ABDON (algae seed) (68% and 56% versus 19%). This result, which is logical because of no or minimal presence of algae in the treatment facility, was similarly observed for the Fargo WWTF but not as dramatic.

Due to the short SRT in HPO-AS, nitrification was limited leading to ammonia build-up as ammonification of DON occurred. The longer SRT in the MBBR resulted in higher nitrification and lower ammonification because nitrifying bacteria outcompeted ammonifying bacteria for oxygen. Additional factors that might have masked the removal of DON and BDON in the MBBR included low carbon to nitrogen ratio, production of soluble microbial products, and/or hydrolysis of particulate organic matter entrapped in MBBR media. BDON and ABDON slightly increased after the polishing pond which could be due to changes in DON characteristics induced by environmental processes such as photodegradation (Bronk et al., 2010). It should be noted that chlorination and dechlorination were not practiced during the sampling period. The levels of final effluent BDON and ABDON for the two WWTFs studied were in a similar range.

The overlapping portion of DON and DON utilizable exclusively by algae and bacteria for samples from the Moorhead WWTF were calculated and the results are presented in Table 2. The overlapping DON values were substantially lower for the Moorhead WWTF compared to those for the Fargo WWTF particularly for the first three sampling locations. There is no trend in the overlapping DON along the

treatment train. DON utilizable exclusively by bacteria and by algae was higher for the Moorhead WWTF. DON utilizable exclusively by algae was comparable with that by bacteria for the last two sampling locations and was 24% and 27% of ABDON exerted by bacteria + algae seed. This result reiterates the importance of algae as wastewater treatment organisms especially for DON removal.

DON biodegradability (BDON/DON before incubation) and DON bioavailability (ABDON/DON before incubation) data are presented in Fig. 6b. The DON biodegradability and bioavailability of the Moorhead WWTF were much lower than those of the Fargo WWTF. This is also true for the final effluent and is mainly due to higher DON for the Moorhead WWTF but comparable BDON and ABDON between the two WWTFs. Increases in DON biodegradability and bioavailability between the last two locations were due to slight increases in BDON and ABDON as discussed above but a slight decrease in DON (Fig. 5b).

Studies have demonstrated that effluent DON from activated sludge WWTFs is both biodegradable and bioavailable (Khan et al., 2009; Bronk et al., 2010; Filippino et al., 2011). The present study showed that a significant portion of effluent DON is biodegradable and/or bioavailable regardless of the type of wastewater treatment system in use (trickling filter or activated sludge). The results from this study elucidated the role of several types of treatment processes in the removal of these two fractions of DON, which significantly varied with the type of inocula used in the analytical method. The use of all three types of inocula (bacteria, algae, and algae and bacteria) and the estimation of overlapping DON have provided a greater understanding of the symbiotic relationship between these two groups of organisms. Understanding the performances of treatment processes for their removal of BDON and ABDON along with the relationship between these DON fractions and operational parameters such as SRT would help in the design and operation of treatment facilities. However, varying the operating condition(s) at full-scale treatment facilities for a study to gain such knowledge is not easy to do due to the effluent regulation. A laboratory-scale study is currently in progress to determine the effect of SRT on the biodegradability of DON and will soon be reported.

3.3. BioWin model simulations

DON and BDON profiles from the BioWin model simulations along the Fargo WWTF are presented in Figs. 3b and 4a, and those along the Moorhead WWTF are presented in Figs. 5b and 6a. Calibrated influent fractionation and kinetic parameters for both WWTFs are summarized in Table 3. Simulation results for DON from the model calibrated by Simsek et al. (2012) matched well with the Fargo WWTF data collected in the present study, while the BDON after nitrification filters was slightly under-predicted. Model calibration with the Moorhead WWTF data matched fairly well for both DON and BDON along the treatment facility.

Several differences were identified for model calibration parameters between the Fargo and Moorhead WWTFs. The calibrations indicated that Moorhead wastewater influent has considerably greater soluble unbiodegradable TKN, which is defined as non-biodegradable DON or NBDON (Simsek et al., 2012), than that of Fargo (Table 3). Except ammonification

Table 2 – Overlapping DON and DON utilizable exclusively by algae and bacteria for samples from Moorhead WWTF.

Sample location	Overlapping DON (mg/L)	DON in mg/L utilizable exclusively by	
		Bacteria	Algae
After primary clarifier	0.69	3.59	1.42
After secondary clarifier	0.53	2.09	1.03
After MBBR	0.92	0.67	0.51
Final effluent	1.03	0.79	0.67

Table 3 – Calibrated influent fractionation and kinetic parameters.

Parameter	Default	Fargo WWTF ^a	Moorhead WWTF
1. Influent fractions			
F_{us} – Unbiodegradable soluble COD [g COD/g of total COD]	0.05	0.067	0.067
F_{up} – Unbiodegradable particulate COD [g COD/g of total COD]	0.13	0.16	0.16
F_{na} – Ammonia as a fraction of TKN [g $\text{NH}_3\text{-N}$ /g TKN]	0.66	0.74	0.765
F_{nox} – Particulate organic nitrogen [g N/g Organic N]	0.5	0.005	0.005
F_{nus} – Soluble unbiodegradable TKN [g N/g TKN]	0.02	0.065	0.115
F_{upN} – N:COD for unbiodegradable particulate COD [g N/g COD]	0.035	0.001	0.001
2. Kinetic parameters			
Ammonia oxidizing bacteria			
Maximum specific growth rate [1/d]	0.9	1.2	0.9
Substrate half saturation [mg N/L]	0.7	0.7	0.7
Nitrite oxidizing bacteria			
Maximum specific growth rate [1/d]	0.7	1	0.7
Substrate half saturation [mg N/L]	0.1	0.1	0.1
Ordinary heterotrophic organisms			
Hydrolysis rate [1/d]	2.1	0.5 [1] 1.2 [2]	2.1
Ammonification rate [L/(mg N d)]	0.04	0.01 [1] 0.04 [2]	0.024
[1] First-stage trickling filters.			
[2] Second-stage trickling filters.			
a Values obtained from Simsek et al. (2012).			

rate, default kinetic and stoichiometric parameters were used during the model calibration with the Moorhead WWTF data, while several kinetic parameters needed to be adjusted during the calibration with the Fargo WWTF data. The maximum specific growth rates for ammonia and nitrite oxidizing bacteria were higher for the TF system compared to the HPO-AS and MBBR system. Hydrolysis and ammonification rates were higher for the second stage trickling filters between the two stages of the TF system. A hydrolysis rate for the HPO-AS and MBBR system was higher than those of the TF system. This is likely because of better oxygen transfer in the HPO-AS and MBBR system.

ABDON fractions (ABDON-algae alone and ABDON-algae + bacteria) were not simulated in the present study because BioWin[®] currently does not have the capability to simulate these fractions. Nitrogen fractionation in BioWin[®] includes specific terms for biodegradable portions of organic nitrogen both in soluble and particulate forms. The processes that control the conversion of these organic forms in the model are hydrolysis and ammonification. Ammonification or mineralization is a bacterial driven process. Future studies are necessary to understand the kinetics of algal growth on bioavailable fractions of DON, which would be helpful in simulating the fate of these fractions through WWTFs.

4. Conclusions

A comprehensive study was conducted to investigate the fate of BDON and ABDON through the treatment trains of two different WWTFs, one with activated sludge + MBBR process (Moorhead WWTF) and the other one with a two-stage trickling filter system (Fargo WWTF). A combination of bacterial and algal seeds always provided the highest DON reduction (ABDON exertion) compared to bacterial only seed

and algal only seed and therefore should be used to determine the worst case scenario of the impact of effluent DON on receiving waters. Both biological processes studied were not distinctively different in their abilities for BDON and ABDON removal efficiencies which were substantial. However, the TF plant was better in DON reduction than the AS plant resulting in effluent with higher DON bioavailability and biodegradability (ABDON/DON and BDON/DON ratios). A certain fraction of wastewater DON was utilizable by algae only suggesting the use of algae as an additional group of organisms in the treatment train particularly at the tertiary level in order to minimize reactive DON load and in turn reduce eutrophication potential in receiving water environment. Elucidating wastewater DON chemical composition particularly for strictly utilizable and overlapping fractions to further understand fate of DON in wastewater facilities and receiving waters and possible control strategies is recommended for future study.

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REFERENCES

- APHA, 2005. Standard Methods for the Examination of Water and Wastewater, twenty-first ed. American Public Health Association, Washington, DC.

- Bronk, D.A., See, J.H., Bradley, P., Killberg, L., 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeochemistry* 4, 283–296.
- Bronk, D., Roberts, Q.N., Sanderson, M.P., Canuel, E.A., Hatcher, P.G., Mesfioui, R., Filippino, K.C., Mulholland, M.R., Love, N.G., 2010. Effluent organic nitrogen (EON): bioavailability and photochemical and salinity-mediated release. *Environmental Science and Technology* 44 (15), 5830–5835.
- Carlsson, P., Graneli, E., Segatto, A.Z., 1999. Cycling of biologically available nitrogen in riverine humic substances between marine bacteria, a heterotrophic nanoflagellate and a photosynthetic dinoflagellate. *Aquatic Microbial Ecology* 8 (1), 23–36.
- Filippino, K.C., Mulholland, M.R., Bernhardt, P.W., Boniello, G., Morse, R., Semchecki, M., Marshall, H., Love, N.G., Roberts, Q., Bronk, D.A., 2011. The bioavailability of effluent-derived organic nitrogen along an estuarine salinity gradient. *Estuaries and Coasts* 34, 269–280.
- Khan, E., Awobamise, M., Jones, K., Murthy, S., 2009. Method development for measuring biodegradable dissolved organic nitrogen in treated wastewater. *Water Environment Research* 81 (8), 779–787.
- Murthy, S., Jones, K., Baidoo, S., Pagilla, K., 2006. Biodegradability of dissolved organic nitrogen: adaptation of the BOD test. In: *Proceedings of the Water Environment Federation 79th Annual Technical Exhibition & Conference*, Dallas, Texas.
- Parkin, G.F., McCarty, P.L., 1981. Production of soluble organic nitrogen during activated sludge treatment. *Journal of Water Pollution Control Federation* 53 (1), 99–112.
- Pehlivanoglu, E., Sedlak, D.L., 2004. Bioavailability of wastewater-derived organic nitrogen to alga *Selenastrum capricornutum*. *Water Research* 38 (14–15), 3189–3196.
- Pehlivanoglu-Mantas, E., Sedlak, D.L., 2006. Wastewater-derived dissolved organic nitrogen: analytical methods, characterization and effects – a review. *Critical Review in Environmental Science and Technology* 36 (3), 261–285.
- Simsek, H., Kasi, M., Wadhawan, T., Bye, C., Blonigen, M., Khan, E., 2012. Fate of dissolved organic nitrogen in two stage trickling filter process. *Water Research* 46 (16), 5115–5126.
- Sattayatewa, C., Pagilla, K., 2008. Nitrogen species measurement in low total nitrogen effluents. In: *Proceedings of the Water Environment Federation 81st Annual Technical Exhibition & Conference*, Chicago, Illinois.
- Sattayatewa, C., Pagilla, K., Pitt, P., Selock, K., Bruton, T., 2009. Organic nitrogen transformations in a 4-stage Bardenpho nitrogen removal plant and bioavailability/biodegradability of effluent DON. *Water Research* 43 (18), 4507–4516.
- Uemoto, H., Ando, A., Saiki, H., 2000. Effect of oxygen concentration on nitrogen removal by *Nitrosomonas europaea* and *Paracoccus denitrificans* immobilized with tubular polymeric gel. *Journal of Bioscience and Bioengineering* 90 (6), 654.
- Urgun-Demirtas, M., Sattayatewa, C., Pagilla, K.R., 2008. Bioavailability of dissolved organic nitrogen in treated effluents. *Water Environment Research* 80 (5), 397–406.
- UTEX, 2011. The Culture Collection of Algae: Web interface. Retrieved on July 20, 2011. <http://www.sbs.utexas.edu/utex/mediaDetail.aspx?mediaID=29>.